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IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF	:
MASAHIRO KAKEHI, ET AL.	: EXAMINER: E. SLOBODYANSKY
SERIAL NO: 10/798,339	:
FILED: MARCH 12, 2004	: GROUP ART UNIT: 1652

FOR: METHOD FOR PRODUCING NUCLEOTIDE BY FERMENTATION

APPEAL BRIEF

COMMISSIONER FOR PATENTS
ALEXANDRIA, VIRGINIA 22313

SIR:

This is an appeal from the Final Rejection of the claims dated August 27, 2007.

I. REAL PARTY IN INTEREST

The real party in interest is Ajinomoto Co, Inc.

II. RELATED APPEALS AND INTERFERENCES

Appellants, Appellants' legal representative and their assignee are not aware of any appeals or interferences which will directly affect or be directly affected by or having a bearing on the Board's decision in this appeal.

III. STATUS OF THE CLAIMS

The appealed claims are Claims 9 and 11-14, the only claims in the case.

IV. STATUS OF AMENDMENTS

No amendments have been filed subsequent to the mailing of the Final Rejection on August 27, 2007.

V. SUMMARY OF THE CLAIMED SUBJECT MATTER

A copy of the appealed claims is submitted in the attached Claims Appendix.

As set forth in Claim 9, the present invention is directed to a method for producing nucleoside 5′-phosphate esters [page 3, lines 19-22 of the specification], comprising the steps of culturing a bacterium belonging to *Escherichia coli* having an ability to produce nucleoside 5′-phosphate ester [page 4, lines 9-12 of the specification], in which expression of *ushA* gene and *aphA* gene is decreased as compared to a wild type strain [page 4, lines 12-13 of the specification] by mutating or disrupting the *ushA* gene and the *aphA* gene [page 4, lines 17-21 of the specification], in a medium to produce and accumulate nucleoside 5′-phosphate ester in a medium [page 4, lines 14-15 of the specification], and collecting the nucleoside 5'-phosphate ester from the medium [page 4, lines 15-16 of the specification], wherein the nucleoside 5′-phosphate ester is selected from the group consisting of inosine 5′-phosphate ester and guanosine 5′-phosphate ester [page 4, lines 23-25 of the specification], and wherein the 5'-nucleotidase activity in the periplasm is substantially eliminated [see page 31, lines 12-15 of the specification].

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

1. Whether Claims 9, 13 and 14 are unpatentable under 35 U.S.C. §103(a) over Thaller et al. alone or in view of Cowman et al.

2. Whether Claims 9 and 11-14 are unpatentable under 35 U.S.C. §103(a) over Thaller et al. alone or in view of Cowman et al. and Matsui et al.

VII. ARGUMENT

The present invention is based on the discovery that decreasing expression of *ushA* gene and *aphA* gene by mutating or disrupting the *ushA* gene and the *aphA* gene substantially eliminates the 5'-nucleotidase activity in the periplasm. As a result, inosine 5′-phosphate ester and guanosine 5′-phosphate ester are produced at improved yields as compared to wild-type *Escherichia coli*.

Thaller et al. describe the cloning of the *ushA* gene and the 5'-nucleotidase activity thereof. See the Abstract. There is no description that decreasing expression of the gene would lead to enhanced production of nucleoside 5′-phosphate esters.

Cowman et al. identify the *aphA* gene and describe the dephosphorylation activity of that enzyme. See the Abstract and pages 195 and 196. In particular, the reference describes a hybrid plasmid encoding the enzyme. Cells transformed with the plasmid synthesize about 20 times the normal amount of the enzyme. Cowman et al. explain at page 285 of the reference that the plasmid may useful in cloning applications. There is no teaching in the reference that decreasing expression of the gene would lead to enhanced production of nucleoside 5′-phosphate esters.

Matsui et al. disclose a process for producing purine nucleosides via fermentation using a modified PRPP amidotransferase. See the Abstract. There is no teaching in the reference that decreasing expression of the *ushA* gene and *aphA* gene would lead to enhanced production of nucleoside 5′-phosphate esters.

Thaller et al. alone or in combination with Cowman et al. and Matsui et al. fail to suggest the claimed method. Neither Thaller et al. or Cowman et al. suggest disrupting expression of the *ushA* and *aphA* genes, respectively, to for the purpose of preparing nucleoside 5′-phosphate esters. In fact, Thaller et al. actually suggests increasing expression of *aphA*:

> ...the relatively broad substrate profile shown by the *E. coli* AphA enzyme would suggest that it (and likely also the other enterobacterial homologs) might function as a periplasmic broad-spectrum dephosphorylating enzyme able to scavenge both 3'- and 5'-nucleosides and also additional organic phosphomonoesters. [Thaller et al., page 198, first column, lines 1-7.]

Matsui et al. relates to PRPP amidotransferase. That reference is completely silent with respect to decreasing expression of he *ushA* gene or the *aphA* gene.

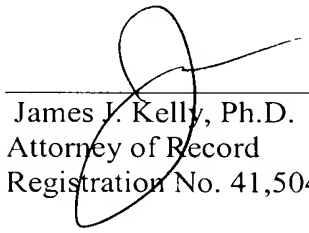
Thus, there is no suggestion in the references to decrease expression of the *ushA* gene and the *aphA* gene by mutation or disruption of the same, for the purpose of producing nucleoside 5′-phosphate esters as claimed.

Moreover, the cited references, alone or in any combination, fail to suggest that decreasing expression of *ushA* gene and *aphA* gene by mutating or disrupting those genes would substantially eliminate the 5'-nucleotidase activity in the periplasm, as claimed. Therefore, that result would not have been predicted from the cited references and establishes the non-obviousness of the claimed method.

In view of the foregoing, the claimed method is not obvious in view of Thaller et al. Cowman et al. and Matsui et al. Accordingly, reversal of the Examiner's rejections of the appealed claims under 35 U.S.C. §103(a) is requested.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.



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Claims Appendix

Claims 1-8: (Canceled).

9. A method for producing nucleoside 5′-phosphate ester, comprising the steps of culturing a bacterium belonging to *Escherichia coli* having an ability to produce nucleoside 5′-phosphate ester, in which expression of *ushA* gene and *aphA* gene is decreased as compared to a wild type strain by mutating or disrupting the *ushA* gene and the *aphA* gene, in a medium to produce and accumulate nucleoside 5′-phosphate ester in a medium, and collecting the nucleoside 5'-phosphate ester from the medium, wherein the nucleoside 5′-phosphate ester is selected from the group consisting of inosine 5′-phosphate ester and guanosine 5′-phosphate ester, and wherein the 5'-nucleotidase activity in the periplasm is substantially eliminated.

Claim 10: (Canceled).

11. The method according to Claim 9, wherein the bacterium is further transformed with the mutant *purF* gene of *Escherichia coli* coding for PRPP amidotransferase in which the lysine residue at position 326 is replaced with a glutamine residue.

12. The method according to Claim 11, wherein the bacterium is further transformed with a guaBA operon of *Escherichia coli*.

13. The method according to Claim 9, wherein the nucleoside 5′-phosphate ester is inosine 5′-phosphate ester.

14. The method according to Claim 9, wherein the nucleoside 5′-phosphate ester is guanosine 5′-phosphate ester.

Evidence Appendix

None

Appeals and Related Proceedings Appendix

None